EXTRACTION OF KAPPA CARRAGEENAN FROM LOCAL EDIBLE SEAWEED

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ABSTRACT

Kappa carrageenan is a polysaccharides which can be extracted from red seaweed such as *Eucheuma cottoni* species. This study report the extraction of kappa carrageenan from local edible seaweed. The effect of various concentrations of extracting solvent (KOH), time and temperatures on kappa carrageenan properties was studied. The KOH solutions (concentrations 0.03,0.05, 0.1M) were used as extracting solvent. Extraction process was carried out in oil bath with a constant ratio of seaweed weight to solvent volume (1:20 g/ml) at temperatures (80, 90, 100 ℃) for 1,2,3, and 4 hours. During extraction process the samples were stirred using magnetic bar and hot plate stirrer for 10 minutes for every half an hour. Then for the isolation process, the potassium chloride (2% w/v) was used to make the kappa carrageenan become precipitated. The precipitated carrageenan were left to dried at room temperature and were kept in desiccators until further analysis. Higher KOH concentration lead lower sulphate content and higher gel strength of extracted carrageenan. In terms of yield the 0.05M KOH, sample C2 (0.05M, 80℃ , 3h) showed the highest yield, while to get highest gel strength the best parameter is L3 (0.1M,100℃, 4h)

ABSTRAK

Kappa karagenan merupakan polisakarida yang diekstrak daripada rumpai laut merah dari spesies *Eucheuma cottoni* . Kajian ini menunjukkan kaedah pengekstrakan kappa karagenan daripada rumpai laut tempatan. Kajian ini melibatkan kesan penggunaan kepekatan larutan pengekstrakan (KOH), masa dan suhu yang berbeza terhadap ekstraks kappa karagenan yang terhasil. Larutan pengekstrakan yang digunakan ialah kalium hidroksida (kepekatan : 0.03M, 0.05M, 0.1M) . Proses pengekstrakan dilakukan menggunakan minyak dengan nisbah berat rumpai laut dan larutan pengekstrakan yang sama (1:20 g/ml) pada suhu (80, 90, 100 ℃) selama 1,2,3, dan 4 jam. Semasa proses pengekstrakan, sampel akan dikacau selama 10 minit bagi setiap setengah jam. Selepas itu, Kalium klorida (2% w/v) digunakan ketika proses pengasingan kappa karagenan daripada jenis karagenan yang lain. Mendapan kappa karagenan ini kan dibiarkan kering pada suhu bilik dan disimpan di dalam desiccators sehingga analisa seterusnya. Dari hasil kajian menunjukkan bahawa kepekatan kalium hidroksida yang tinggi membawa kepada pengurangan kandungan sulfur, dan peningkatan kekuatan gel yang terhasil. Dari segi hasil pengekstrakan, parameter 0.05M, sample C2 (0.05M, 80℃ , 3h) adalah yang tertinggi sementara untuk menghasilkan gel yang kuat parameter yang terbaik ialah L3(0.1M, 4jam, 100℃).

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LIST OF ABBREVIATIONS

W_1	weight of dried carrageenan of Equation 1
W_2	weight of white ash of Equation 1
w_0	initial weight of sampel of Equation 2
w_t	weight of swollen gels of Equation 2

LIST OF ABBREVIATIONS

KOH	Potassium hydroxide
KCL	Potassium chloride
PDA	Potato Dextrose Agar
TPC	Total Plate Count
$BaCl_2$	Barium chloride
$BaSO_4$	Barium sulphate

1 INTRODUCTION

1.1 Motivation and statement of problem

Many ingredients are added to various food systems in order to provide a wide selection of products for the consumer to choose from. Food hydrocolloids or food gums are added to food systems for numerous reasons, mainly to modify the texture, increase the stability, or reduce the fat or calories of a product. Specifically, food hydrocolloids are used to thicken, gel, control syneresis, stabilize an emulsion or suspension, function as a coating, and bind water. Use of food hydrocolloids continues to increase with recent development of low-fat and reduced-fat products as well as in the formulation of products in need of thermal or freeze-thawing stability. There are a variety of hydrocolloids on the market, including those derived from plants or seaweed, and those produced by microorganisms. Increasing numbers of products in the form of a blend of hydrocolloids are now available commercially for specific areas of applications such as reduced gelling points or increased viscosity. However, only limited information exists in the literature that fully characterized their applicability in mixture. It has been demonstrated that the structure of the hydrocolloid, including the type and number of monosaccharide backbone as well as the type, number, and distribution of side units, determines its characteristics and behavior in solutions. Moreover, the net charges on the polymeric side chains also play an important role in their functionality as well. In general, hydrocolloids have a sugar backbone that contains protruding substituents such as esters, sulphates, or additional sugars. Hydrocolloids available for food applications are either neutral or negatively charged (Sadar, 2004) .

Carrageenan, the third important hydrocolloid in the world after the starch and gelatine, occurs as matrix material in various species of red seaweeds (*Rhodophyta*). It is a negatively charged hydrocolloid derived from red seaweed plants, has been widely used in ice cream, chocolate milk, jellies, sauces and dessert gels (Sadar, 2004). Nowadays, the global market is based on three types of carrageenan, namely kappa carrageenan, lambda carrageenan and iota carrageenan. Kappa carrageenan forms strong , rigid gels when combined with potassium ions in the mixture while iota forms weak elastic gel, the lambda carrageenan is non-gelling type (Mustapha *et al*., 2011).

Common use of carrageenan is as a food additive, but the researcher now has increased attention of its value to the pharmaceutical industries, as an alternative choice of making vegetarian capsule, which is animal free.

Nowadays, gelatine is one of the widely used raw material in food (thickening agent), pharmaceutical (gelatine capsule) as well as cosmetics product (creams, lotion). In the pharmaceutical industry, gelatin is used as hard and soft capsules, sugar-coated pills, tablets, serum substitute and vitamin encapsulation. The use of gelatine an all rounder. It will increase the chance of Muslims and vegetarian to exposed to animal based gelatin (Sahilah *et al*.,2012). It can be seen that, kappa carrageenan has the same characteristic with gelatin in terms of strength and elasticity. Thus it is the most suitable substance to use as alternative gelatine.

A good source of kappa carrageenan is *Eucheuma Cottoni*, which have been harvested commercially in Sabah, Malaysia (Distantina *et al*., 2011). Hence this research problem statement is to extract kappa carragenan which is a good source to produce strong and rigid gel with high yield by modifying various parameters, as alternative to replace gelatine is high demand among Muslim and vegetarian. To enhance the gelling properties it needs to be mixed with alkaline solvent, normally potassium hydroxide (KOH) will be used as a extracting solvent., a well known method to extract the carrageenan. Furthermore, some physico-chemical of the seaweeds is also being investigated in order to evaluate their potential use for other product.

1.2 Objective

The following is the objective of this research:

- To study the operating conditions of extracting solvents (KOH) on kappa carrageenan from local seaweed.

1.3 Scope of this research

The following are the scope of this research:

i) To investigate the effect of different concentrations of extracting solvent (KOH) (concentrations: 0.03, 0.05 and 0.1 M) that will be used to extract kappa carrageenan

ii) To investigate the effect of different temperature (80, 90 and 100°C) on kappa carrageenan

iii) To investigate the effect of different extracting time (1h,2h,3h 4h) on kappa carrageenan

1.4 Main contribution of this work

This study will provide data for the best operating condition of extraction of obtaining kappa carrageenan that can be used for further research. Thus help to increase seaweed processing as there are high demand of kappa carrageenan in the market especially in food and pharmaceutical industries. Other than that, it indirectly will help people to cultivate socio-economy development in rural area, particularly in Sabah.

2 LITERATURE REVIEW

2.1 Seaweeds

Marine macroalgae, also known as seaweeds, are not true plants, as they does not have flowers or any clearly marked steam or leaves. They also does not have true roots but held fast which does not absorb food but simply attaches the plant firmly to a stone or rock. In some stage in their life cycles , all seaweeds are unicellular, as spore or zygotes, and may be temporarily plank tonic (Fard, 2009).

Seaweeds are differentiate by their pigmentation, morphology, anatomy and nutritional composition (Benjama *et al.*, 2011). There are over 9,000 species of seaweeds which can be catagorized into three major types, they are : red (*Rhodophyta*), brown (*Phaeophyta*) and green (*Chlorophyta*) (Figure 2.1). Among these three types, red seaweeds are the most rich group (6,000) followed by brown (2,000) and lastly green (1,200) (Fard, 2009).

Figure 2. 1: Three different types of seaweed (a) *Rhodophyta* (b) *Phaeophyta* (c) *Chlorophyta*

Source: CP Kelco ApS , 2001

Around 250 seaweeds species have been commercially utilized worldwide and about 150 species are favourably consumed as human food. In most Asian countries, seaweeds have been consumed since ancient times as source of human food, animal feed and fertilizer. High consumption of brown (66.5%), red (33%) and green (5%) is in Japan and China compared to other Asian countries. While in Western countries seaweed polymers are used as a source of hydrocolloids, thickening, and gelling agents in food and pharmaceutical industries (Benjama *et al.*, 2011) .

According to McHugh (2003) other countries such as Republic of Korea, the United States, South America, Ireland have showed increasing in terms of consumption, production and marketing of seaweeds. About one million tonnes of wet seaweeds were harvested in 35 countries as a source of food, polysaccharides, fertilizer, fuel and cosmetics annually. However in Malaysia, seaweeds are not common to consume as a food. Seaweeds is only consumed in certain coastal areas especially along the east coast of peninsula Malaysia, where it is used in salad dish (Fard, 2009).

Seaweeds is a good source of polysaccharides, protein , vitamin, minerals and dietry fiber (Cox *et al*.,2011). Due to that , they have been recognized to give a huge beneficial to human as well as animal health. The content of nutrients in seaweeds are greatly depending on the species, habitats, maturity and environmental conditions (Benjama *et al.*, 2011) .

2.2 Polysaccharides as stabilizing agents

Polysaccharides are well known additives in the food industries as their had the capabilities of gel forming and thickening agent. They are commonly extracted from seaweeds which yield carrageenan. In market, there are types of carrageenan namely kappa, iota and lambda. Others source of polysaccharides that used as stabilizing agents are dextran and xanthan which are produced by microbial fermentation (Glickman, 1969). There are some stabilizer that are derived by chemical modification of natural products such as low-methoxy-pectin, methylcellulose and carboxymethyl starches (Glickman, 1969).

These gums are used to stabilizer or to improved the body of such products as jams, sauce and mayonnaise is due to their thickening affect. Gel formation which is a property of relatively few polysaccharides is the basis of their application as gelling agents in products, for examples, chocolate milk, puddings and mousses. The properties and applications of gums are reviewed by Whistler and Be Miller (1959) and Glickman (1969). In this thesis special attention will be paid to the properties of kappa carrageenans and to the mechanism on which their application in the food and pharmaceutical industries.

2.3 Carrageenan

A. Origin and chemical composition

The carrageenan is a water soluble hydrocolloid which is come from seaweed. The content of carrageenan can be vary based on type of species and seasonally and its can be found in the cell wall of the seaweed from which it is derived. It is harvested from various regions of the world including the northern part of the US, Philippines, Indonesia, Chile, Argentina, Morocco and France. There are 3 species that are commonly used commercially, they are *Eucheuma, Chondrus Crispus* and *Gigartina.* The main species of *Rhodophycae* used in commercial production of carrageenan are *Eucheuma cottonii* and *E.spinosum*. these are spiny bushy plants, approximately 50cm in high, which grow on reefs and in shallow lagoons (Philips *et al*., 2000).

Carrageenan is polymer consists of repeating liner chains of galactans units with negative charge of numerous ionic sulphate half-ester groups. The repeat unit is a dimer of galactose and anhydogalactose linked by a beta 1, 4 glycosidic linkage (Figure 2.2) . These dimmers are then linked together through alpha 1,3 glycosidic linkage. This secondary structure expect the chair conformation to minimize steric repulsions caused by axial components (Fisher, 2009).

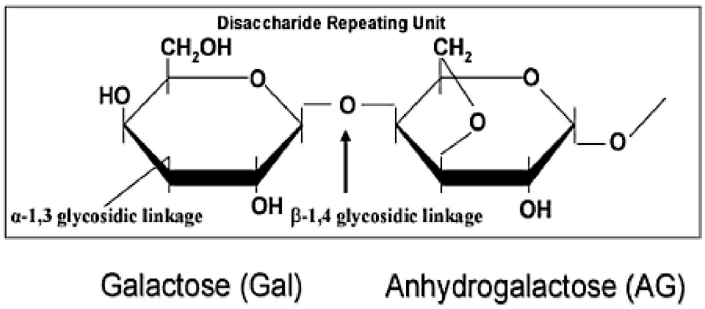


Figure 2. 2:1, 4 glycosidic linkages between galactose and anhydrogalactose monomers in carrageenan

Source: Fisher , 2009

B. Types of Carrageenan

There are three forms of carrageenan, namely kappa, iota and lambda. Each carrageenan is determined by the number and position of the sulphate groups on each sugar and the presence or absence of the 3,6 anhydro group on the B monomer. The 3,6 anhydro group promotes ∝ helix formation which is important for gelling. This due to increase flexibility that promotes a random coil structure.

Figure 2.3 shows kappa carrageenan contains one sulphate group per repeat dimer, located on the O-3 galactose ring. It has structure is right-handed double helix of parallel chains, due to this structure, kappa carrageenan forms durable thermoreversible gels by itself. In the presence potassium salts it forms even more strong and rigid gels.

A right handed double helix of parallel chains contains two sulphate groups per repeat dimer in iota carrageenan located one on each of the sugar units. It will form strong, elastic , thermoreversible gels with limited syneresis. When it mix with calcium forms ionic bridges between iota carrageenan chains, yielding gels with increased gelling and melting temperatures. While for lambda carrageenan, non-gelling carrageenan , it does not have 3,6 anhydro group necessary to form the double helix. It just consists of three sulphated groups with repeat dimer units of D-galactose-2-sulphate-D-galactose-2, 6-disulphate. The molecular structures of kappa, iota and lambda carrageenan was shown in Figure 2.3 (Fisher, 2009).

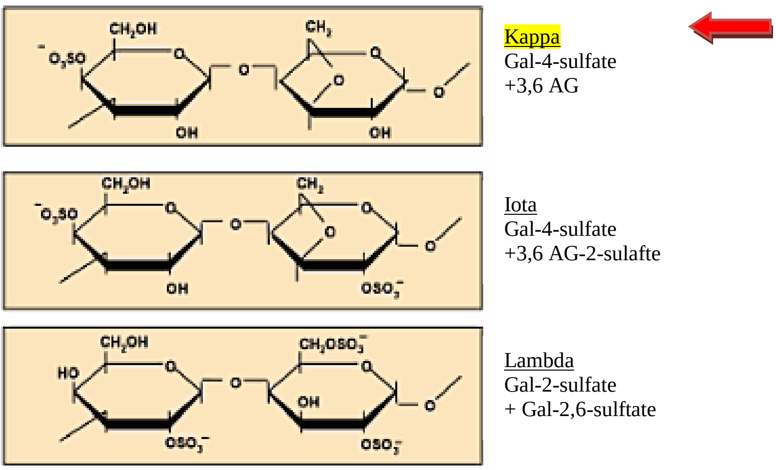


Figure 2. 3: Molecular structures of kappa, iota and lambda carrageenan. Kappa carrageenan (red arrow) is use in this study.

Source: Fisher , 2009

C. Bioactive component in kappa carrageenan

There are several types of bioactive components of seaweed, mainly are polysaccharides, antifungal ,anti-microbial and antioxidant. One of the examples of polysaccharides is carrageenans which is considered as a nutraceutical components (Riouxn *et al.*, 2009). According to Plaza *et al* (2009) sulphated polysaccharides also known as carrageenan contain bioactive component such as anti-viral, anti-tumor, antihyperlipidemia, and anti-coagulant, reduce total and LDL cholesterol.

D. Modes of molecular interaction in kappa carrageenan

1. Gelling

Gelling properties in kappa carrageenan is one of its great properties widely used in food industries. The tertiary structure of the carrageenan type is thought to dictate gelation as local regions of ordered molecular associations aggregate to form a disordered polymer network. Whether gelation can occur is highly dependent on the concentration and type of carrageenan, and generally kappa carrageenan require heat about 80℃ to compeletly solubilized. The polymer chains are released into a colloidal state once it solubilized (Figure 2.4). When the carrageenan solubilized it has the negative charged sulphate groups all along the polymer chain, which stimulate the repulsion that forbid chain folding and intermolecular associations. Intermolecular interaction occur when the cation interaction neutralize the negative sulphate groups. When the carrageenan solution get cools, intramolecular hydrogen bonds stabilize the ∝ - helix conformation in individual carrageenan chains and intermolecular hydrogen bonds stabilize the formation for the double and triple helices between carrageenan chains (Fisher ,2009) .

Gelation of solubilized carrageenan polymers

$\boldsymbol{K^{+}}$ promote ∝-helix associations and double helix formation

$\boldsymbol{Ca^{2+}}$ form ionic bridges between (-OS$\boldsymbol{O_3^-}$)

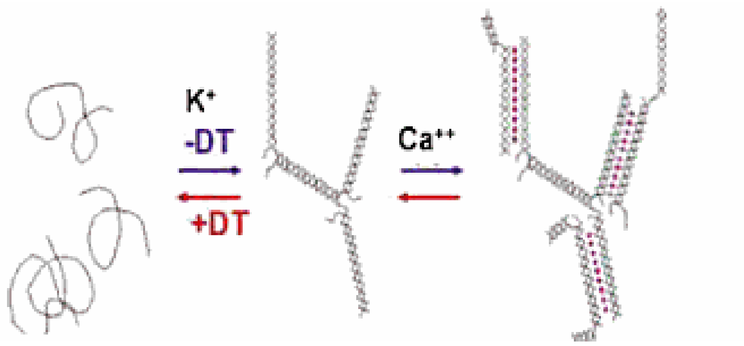
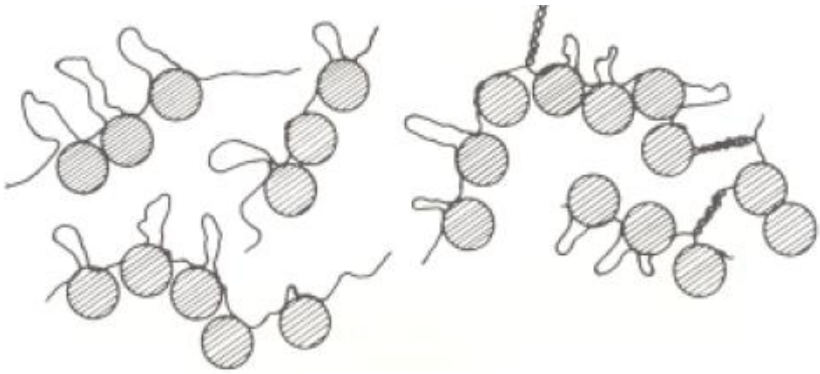


Figure 2. 4:Molecular associations involved in the gelation of carrageenan

Source: Fisher ,2009

As the kappa carrageenan solution cools, firstly the random coil will form double helices before the aggregation occurs (Figure 2.5). When the same gel is reheated, the process reverses, begin with dissociating the aggregates then by restoration of random coil. According to Fisher (2009) extraction of kappa carrageenan at various concentration and temperatures using small angle X-ray scattering found that kappa carrageenan formed two to three double helices during gelation. Eventually this will promotes tighter and more extensive molecular aggregation and yields a rigid.



Solution → Gel

Figure 2. 5: Gelation of carrageenan

Source: Cp Kelco ApS, 2001

Gel promotion is to increase ionic interaction which increase intermolecular associations and change gel transition temperatures. Fisher (2009) found that kappa carrageenan (0.7 to 1.4 %) solution will form weak gels without addition of potassium ions. Kappa carrageenan gelation in various salt showed that $K^+ > Ca^+ >> Na^+$ in effectively increasingly gelling rate, gel melting temperature and gel strength. He also found that thermal transition of kappa carrageenan solution with and without KCl also showed that the conversion temperature from coil to helix and vice versa was higher with addition of KCl. Potassium chloride has the highest effect on gel strength per potassium unit but other potassium salts may be used for taste considerations. Potasium ions also have the effect of incresing the melting and gelling temperatures as illustrated in Figure 2.6 (Cp Kelco ApS, 2001).

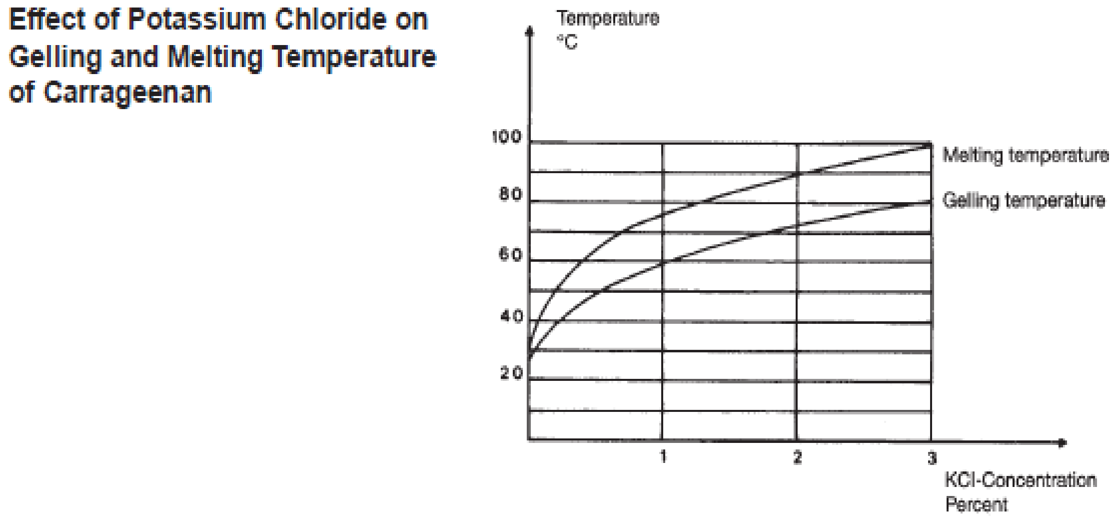


Figure 2. 6:Effect of potassium chloride on gelling and melting temperature of carrageenan

Source: Cp Kelco ApS, 2001

It is believed that barium ions form bridges between adjacent double helices through an electrostatic binding to two adjacent sulphate groups, thus stabilizing and strengthening the network (Figure 2.7). When removing cations which cause gelation of carrageenan from the medium as well as from the carrageenan, a solution of carrageenan is obtained which does not form a gel irrespective of the temperature. As soon as gelling cations are present the carrageenan solution will gel at a specific temperature, the gelling temperature. Thus, the gelling temperature of a carrageenan solution is a function of the concentration of gelling cations present in the system (Cp Kelco ApS, 2001). In this research the barium chloride is used in analysis of yield.

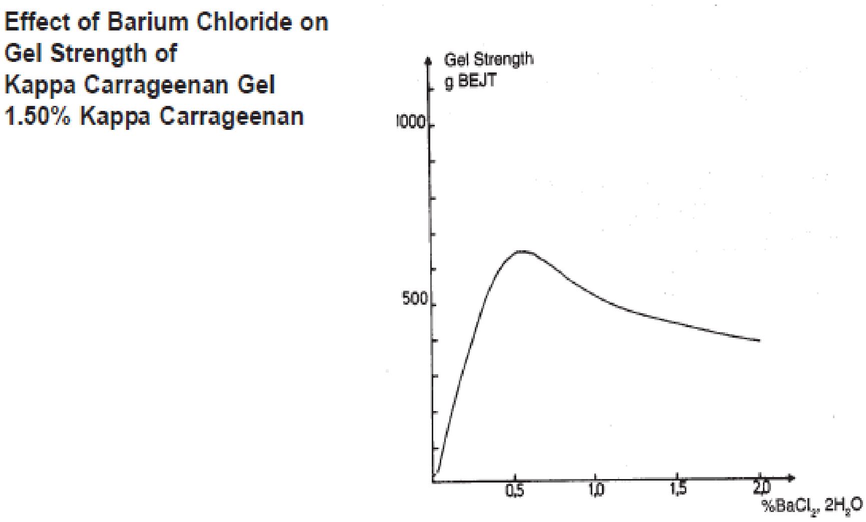


Figure 2. 7:Effect of Barium Chloride on gel strength of kappa carrageenan gel

Source: Cp Kelco ApS, 2001

E. Extraction of kappa carrageenan

Extraction in certain red seaweed of the Rhodophyceae class consists of carrageenan, a sulphated linear polysaccharide. It is used in the food industry as thickening, gelling agent and recently it has been used as excepient in pill and tablets (Distantina *et al*., 2011). Currently, global market based on three types of carrageenan namely kappa carrageenan, iota carrageenan and lambda carrageenan. Among these three, kappa carrageenan will forms strong, rigid gel after combined with potassium hydroxide solution (Mustapha *et al*.,2011). A summary of the solution and gelation properties of carrageenan and its synergy with other materials is given in Table 2.1 (Philips *et al*., 2000).

Table 2. 1:Summary of carrageenan properties

	Lambda	Iota	Kappa
Solubility			
Hot (80°C) water	Soluble	Soluble	Soluble
Cold (20°C) water	All water soluble	Na^+ salt soluble Ca^{++} salt gives thixotropic sols	Na^+ salt soluble Limited swelling of K^+, Ca^{++} salts
Hot (80°C) milk	Soluble	Soluble	Soluble
Cold (20°C) milk	Thickens	Insoluble	Insoluble
Cold milk (TSPP added)	Increased thickening or gelling	Thickens or gels	Thickens or gels
50% sugar solutions	Soluble	Insoluble	Soluble hot
10% salt solutions	Soluble hot	Soluble hot	Insoluble
Gelation			
Effect of cations	Non-gelling	Strongest gels with Ca^{++}	Strongest gels with K^+
Gel texture	–	Elastic	Brittle
Shear reversible gel	–	Yes	No
Syneresis	–	No	Yes
Hysteresis	–	5–10°C	10–20°C
Freeze-thaw stable	Yes	Yes	No
Synergy with locust bean gum	No	No	Yes
Synergy with konjac flour	No	No	Yes
Synergy with starch	No	Yes	No
Salt tolerance	Good	Good	Poor
Stability in acid	Hydrolysis	Hydrolysis of solution, accelerated by heat Gels are stable	
Protein reactivity	Strong interaction increasing at acid pH		Specific reaction with kappa-casein

Source: Philips *et al*., 2000

Figure 2.8 (a) show that kappa carrageenan selects for potassium ions to stabilize the junction zones within the characteristically firm, brittle gel. While, iota carrageenan selects for calcium ions to bridge between adjacent chains to give typically soft elastic gels as shown in Figure 2.8 (b) (Philips *et al*., 2000).

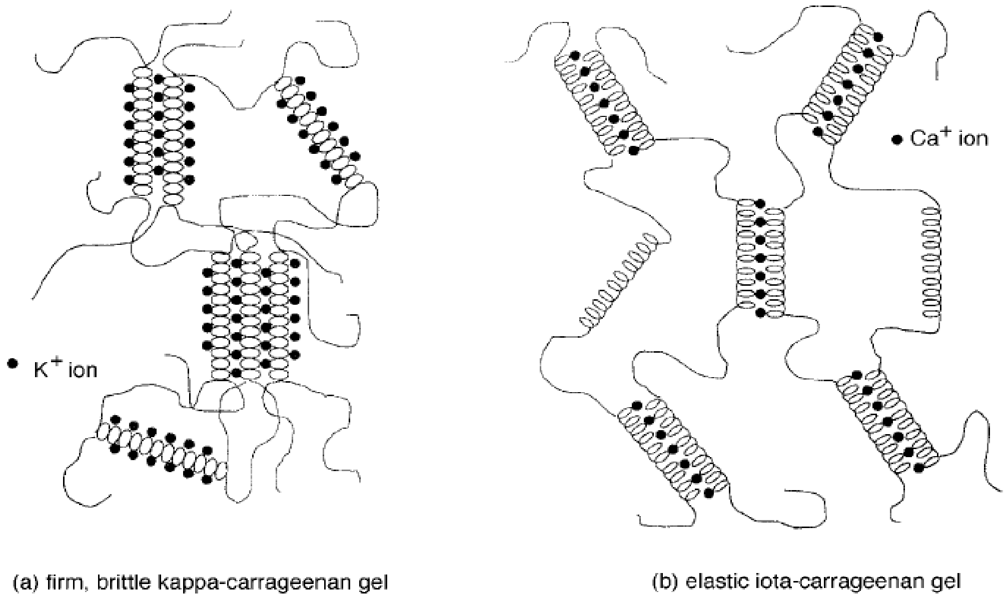


Figure 2. 8:Gelation of kappa and iota carrageenans with cations

Source: Philips et al., 2000

Hot solutions of kappa and iota carrageenans set to form a range of gel textures when cooled to between 40 and 60ºC depending on the cations present. The ionic composition of a food system is crucial for effective utilization of the carrageenan. The seaweeds are usually extracted with alkali at high temperature (Distantina *et al*., 2011). Characteristically, solution contain 1-2 % agar by weight will gel at about 35°C and melt at about 85°C, agar gel produce are strong and brittle (Laurienzo, 2010). The potassium hydroxide (KOH) is used as the extracting solvent because there is a significant relation between gel strength and KOH concentration as illustrated in Figure 2.9 (Distantina *et al*., 2011).

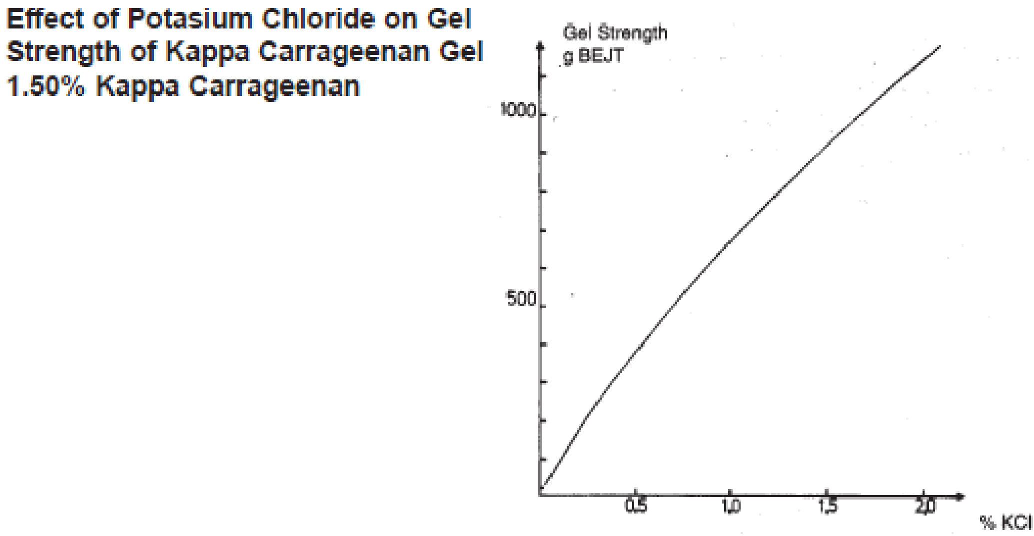


Figure 2. 9:Effect of potasium Chloride on gel strength of kappa carrageenan

Source: Cp Kelco ApS, 2001

Based on other research that comparing of using potassium hydroxide (KOH) and sodium hydroxide (NaOH) as extracting solvent showing that KOH is more preferable because of greater yield and lower losses KOH solution as extracting media compared to NaOH (Tuvikene *et al*., 2006). According to Mustapha *et al*. 2011, selection of $Ca(OH)_2$ as extracted solvent showing no gel formation in all test conditions. By utilizing this research data, it is known that selecting KOH as extracting solvent to extract kappa carrageenan will enhance the gelling properties of seaweed.

2.4 Applications of kappa carrageenan

Carrageenan has been widely used in food industry as it has various uses. The basic carrageenan types may be used individually or mixed with others to form blends. The food applications of carrageenan gum have been divided into dairy based and water based topics (Sadar, 2004). There are also non-food applications of carrageenan (CP Kelco ApS, 2001).